Engineering Terra Preta: A Pilot Study Protocol for Replicating the Iron-Phase Reactor in Degraded Tropical Soils

An Open Experimental Protocol for Soil Scientists, Agricultural Engineers, and Funding Agencies

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March 2026

Companion protocol to: Iron Transformation and Phosphorus Retention in Amazonian Dark Earth: Convergent Geochemical and Microbial Evidence for a Multi-Factorial Reactor Model (Branham, 2026)

Executive Summary

Tropical ferralsols cover approximately one billion hectares globally and support roughly half of all terrestrial agriculture. These soils fix 70–90% of applied phosphate fertilizer on iron and aluminum oxide surfaces, reducing P use efficiency to below 25% and forcing repeated fertilizer application from a non-renewable phosphate rock supply. Reanalysis of 3,689 geochemical samples from Amazonian Dark Earth (ADE) reveals that terra preta achieves 37% phosphorus retention efficiency versus 1% in adjacent ferralsol —a 37× difference—by transforming the iron oxides that normally fix phosphorus into ferrimagnetic phases that no longer immobilize it.

This protocol describes a controlled incubation experiment to test whether the terra preta reactor mechanism can be replicated using three co-applied amendments: biochar (scaffold), agricultural lime (pH intervention), and an iron-reducing microbial inoculant (biological catalyst). The primary endpoint is a measurable increase in magnetic susceptibility coupled with a decrease in exchangeable iron and an increase in desorbable phosphorus—the three-way signature of the reactor. The experiment requires no specialized equipment beyond a portable magnetic susceptibility meter and standard soil chemistry, is designed for completion in 6–12 months, and costs approximately $18,000–$45,000 USD depending on analytical scope (a minimum-viable version measuring only MS, P, pH, and Fe at two timepoints is feasible for under $3,000). All materials, methods, and analysis code are provided as open-access resources. If successful, the protocol establishes a pathway toward an engineered soil amendment that simultaneously enhances food production, reduces fertilizer dependence, and sequesters carbon in stable mineral-organic complexes.

1. The Problem

Phosphorus is the limiting nutrient for roughly 40% of global cropland, and the problem is most severe in tropical ferralsols where iron and aluminum oxides adsorb phosphate ions via ligand exchange, forming increasingly irreversible surface complexes over time. A farmer applying 100 kg P/ha to an untreated ferralsol may recover less than 25 kg in crop uptake; the remainder is locked on oxide surfaces within weeks to months and becomes progressively less desorbable. The standard response—applying more fertilizer—merely saturates more oxide surface area without changing the underlying kinetics. Global phosphate rock reserves are finite and unevenly distributed; at current extraction rates, economically recoverable reserves may be exhausted within 50–300 years depending on estimates.

Amazonian Dark Earth solves this problem. Terra preta retains 37% of total phosphorus in plant-available form versus 1% in adjacent ferralsol—not by adding more P, but by changing the soil's surface chemistry so that deposited P remains in a rapidly exchangeable pool rather than being irreversibly fixed. The mechanism, as described in the companion paper (Branham, 2026), involves a three-component reactor: ash-driven pH elevation, biochar scaffolding, and biologically mediated iron reduction. The iron oxides that normally jail phosphorus are transformed into ferrimagnetic phases (magnetite and maghemite) captured on biochar surfaces, permanently removing them from the P-fixing game.

The question is whether this process, which took centuries in archaeological middens, can be accelerated to agronomic timescales. This protocol is designed to answer that question.

2. Hypothesis

Co-application of biochar, agricultural lime, and a dissimilatory iron-reducing microbial inoculant to ferralsol will, within 6–12 months under controlled moisture and temperature conditions, produce a measurable increase in magnetic susceptibility, a decrease in exchangeable iron, and an increase in desorbable phosphorus relative to single-amendment and untreated controls. This three-way signature replicates the geochemical fingerprint of archaeological terra preta and indicates that the iron-phase reactor mechanism has been initiated.

2.1 Specific predictions

#	Prediction	Measurement	Success criterion
1	Magnetic susceptibility increases in the biochar+lime+inoculant treatment	Portable MS meter (e.g., Bartington MS2) at 0, 3, 6, 12 months	MS increase ≥50% over baseline, significantly greater than single-amendment controls
2	Exchangeable Fe decreases as Fe(III) is reduced and captured on biochar	Mehlich-3 or ammonium oxalate extraction	Fe_{exch} decrease ≥20% relative to biochar-only control
3	Desorbable P increases as Fe oxide sorbent is removed from the P-fixing game	Sequential P fractionation (Hedley method) or anion exchange membrane P	Labile P pool increase ≥30% over lime-only control
4	Iron reducer population declines as substrate is consumed (replicating ADE pattern)	qPCR targeting *Anaeromyxobacter* 16S; optional amplicon sequencing	*Anaeromyxobacter* abundance peaks at 1–3 months, then declines
5	Biochar particles show Fe nanoparticle inclusions	SEM-EDS or TEM on recovered biochar particles (optional)	Fe signal co-located with biochar carbon matrix

Table 1. *Five falsifiable predictions with specific measurement methods and success criteria. Predictions 1–3 are primary endpoints; 4–5 are mechanistic confirmation.*

3. Experimental Design

3.1 Treatments (full factorial)

Treatment	Biochar	Lime	Inoculant	Rationale
T1: Control	—	—	—	Untreated ferralsol baseline
T2: Biochar only	5% w/w	—	—	Tests scaffold alone
T3: Lime only	—	Target pH 6.0	—	Tests pH intervention alone
T4: Inoculant only	—	—	10^7 CFU/g	Tests biology alone
T5: Biochar + Lime	5% w/w	Target pH 6.0	—	Tests scaffold + pH without biology
T6: Biochar + Inoculant	5% w/w	—	10^7 CFU/g	Tests scaffold + biology without pH
T7: Lime + Inoculant	—	Target pH 6.0	10^7 CFU/g	Tests pH + biology without scaffold
T8a: Full Reactor (defined strain)	**5% w/w**	**Target pH 6.0**	**10^7 CFU/g *A. dehalogenans***	**Reactor hypothesis with reproducible inoculant**
T8b: Full Reactor (ADE consortium)	**5% w/w**	**Target pH 6.0**	**10% w/w ADE soil extract**	**Reactor hypothesis with natural microbiome**

Table 2. *Nine-treatment design with dual-track reactor variants. The reactor model predicts that only T8a/T8b (all three components) will produce the three-way geochemical signature. T8b (consortium) may outperform T8a (single strain) by providing the full metabolic network. Single- and two-component treatments demonstrate insufficiency. 4 replicates per treatment = 36 experimental units.*

3.2 Soil substrate

Source a representative ferralsol/oxisol with pH 4.0–5.0 and high Fe oxide content (Fe_{total} ≥5% by weight, typical of tropical red-yellow latosols). The soil should be air-dried, sieved to <2 mm, and homogenized. Baseline characterization: pH (H_2O and KCl), total Fe (XRF or ICP-OES), exchangeable Fe (Mehlich-3), available P (Mehlich-1 or resin-P), total P, organic carbon, magnetic susceptibility, Munsell color, and CEC. A P-fixation assay (24-hour batch sorption isotherm) provides the kinetic baseline.

3.3 Amendment specifications

Biochar: Hardwood biochar pyrolyzed at 500–600°C, particle size 0.5–2 mm, BET surface area ≥200 m^2/g. The pyrolysis temperature ensures aromaticity (scaffold persistence) while retaining surface functional groups (CEC development). Apply at 5% w/w (approximately 50 t/ha equivalent for a 10-cm incorporation depth). Pre-wet biochar to 60% water-holding capacity before mixing to prevent hydrophobic inhibition of colonization.

Lime: Agricultural dolomitic lime ($CaMg(CO_3)_2$), applied at the rate calculated to raise soil pH to 6.0–6.2 (typically 2–5 t/ha equivalent for ferralsol, determined by lime requirement buffer test). This targets the pH range where Fe(III) oxide positive surface charge decreases and microbial iron reduction becomes thermodynamically favorable.

Inoculant (dual-track strategy): We recommend running T8 in two parallel variants. **Track A (defined strain):** *Anaeromyxobacter dehalogenans* strain 2CP-1 (ATCC BAA-258) at 10^7 CFU/g soil. *Anaeromyxobacter* is a microaerophilic Fe(III) respirer that operates under low-oxygen conditions—matching the redox environment of periodically wetted surface soils containing biochar electron shuttles. The defined strain provides reproducibility across laboratories. **Track B (natural consortium):** ADE soil extract at 10% w/w from authenticated terra preta. This captures the synergistic microbiome documented in the companion paper: not only *Anaeromyxobacter* (iron reduction) but also *Paenibacillus* (P-solubilization) and the Firmicutes-dominated community adapted to elevated pH. The consortium approach may outperform the single strain by providing the full metabolic network, including organic acid producers that supply electron donors for iron reduction. If a pure culture is unavailable, Track B alone is acceptable for initial implementations. Running both tracks adds 32 pots but provides a direct comparison between engineering-grade reproducibility and ecological realism.

3.4 Incubation conditions

Fill 2-L pots or microcosm vessels with 1.5 kg treated soil each. Temperature: 28–32°C (greenhouse or growth chamber). Incubate for 12 months with destructive sampling at 0, 1, 3, 6, and 12 months (4 replicates × 5 timepoints = 20 pots per treatment). A reduced design with only 0, 6, and 12 months is acceptable for budget-limited implementations.

Redox oscillation (critical): Maintain a 7-day wetting/drying cycle: wet to 80% water-holding capacity on day 1, allow to drain and dry to 40–50% WHC by day 7, then re-wet. This oscillation is not incidental—it is the reactor's engine. Each wetting phase creates low-O_2 microsites where iron reducers respire Fe(III), mobilizing Fe^{2+}. Each drying phase re-introduces oxygen, causing the mobilized Fe^{2+} to re-precipitate as ferrimagnetic nanoparticles on the nearest available surface—the biochar scaffold. This wet-dry pump replicates the seasonal moisture regime of Amazonian terra firme soils and is likely the mechanism by which ADE accumulated ferrimagnetic minerals over centuries. Permanent waterlogging should be avoided: paddy soil research shows that sustained anaerobic conditions destroy ferrimagnetic minerals rather than building them [11]. The goal is pulsed reduction followed by partial re-oxidation—redox cycling, not permanent reduction.

Carbon source (calibrated): Add 0.5% w/w dried leaf litter at each sampling interval to sustain heterotrophic microbial activity, simulating the organic carbon input that photosynthesis provides in the field. **Caution:** excess labile carbon (glucose, fresh manure) can trigger a microbial bloom that exhausts oxygen so completely that iron reduction overshoots into sulfide territory (FeS, pyrite), which destroys ferrimagnetic minerals and does not help P retention. Use recalcitrant leaf litter (high lignin, e.g., dried hardwood leaves) rather than glucose to provide a slow, sustained carbon supply that matches natural litter input rates.

4. Measurement Protocol

Parameter	Method	Timepoints	Purpose	Est. cost/sample
Magnetic susceptibility	Bartington MS2/MS3 or Terraplus KT-10	0, 1, 3, 6, 12 mo	Primary endpoint: iron transformation	$0 (field meter)
pH (H_2O, KCl)	Electrode, 1:2.5 suspension	0, 1, 3, 6, 12 mo	Track pH trajectory	$2
Exchangeable Fe	Mehlich-3 + ICP-OES	0, 3, 6, 12 mo	Primary endpoint: Fe transformation	$15
Available P	Mehlich-1, resin-P, or Olsen	0, 3, 6, 12 mo	Primary endpoint: P retention	$8
P fractionation	Hedley sequential extraction	0, 6, 12 mo	Labile vs occluded P pools	$40
P sorption isotherm	24-hr batch equilibrium (0–500 mg P/L)	0, 12 mo	Kinetic endpoint: P fixation rate change	$30
Total Fe, P, Mn	XRF or ICP-OES after HF digestion	0, 12 mo	Mass balance	$25
Organic carbon	Walkley-Black or dry combustion	0, 6, 12 mo	Track carbon stability	$10
Munsell color	Munsell chart or spectrophotometer	0, 6, 12 mo	Visual iron phase indicator	$0
CEC / delta pH	pH in H_2O vs KCl	0, 6, 12 mo	Biochar surface oxidation	$4
CEC (full)	Ammonium acetate pH 7, sum of bases	0, 6, 12 mo	Emergent prediction: CEC gain tracks MS	$12
Total N / NH_4^+	Kjeldahl or combustion; KCl-extractable NH_4	0, 6, 12 mo	Emergent prediction: N retention via CEC	$10
16S amplicon (optional)	V4 515F/806R, Illumina	0, 3, 12 mo	Community tracking; within-phylum control (*Haliangium* vs *Anaeromyxobacter* in Myxococcota)	$50
qPCR *Anaeromyxobacter* (optional)	Genus-specific 16S primers	0, 1, 3, 6, 12 mo	Iron reducer dynamics	$15
SEM-EDS (optional)	Scanning electron microscopy + energy dispersive X-ray	12 mo (T8 only)	Fe nanoparticles on biochar	$80

Table 3. *Complete measurement protocol. The first 13 parameters constitute the core protocol (~$18,000 for 36 units × 5 timepoints). CEC and N measurements test emergent predictions from the companion paper (Section 6.4). Optional microbial and imaging analyses add ~$10,000–$25,000.*

5. Statistical Analysis Plan

The primary analysis is a two-way ANOVA (treatment × time) on each of the three primary endpoints (MS, Fe_{exch}, P_{avail}), with Tukey HSD post-hoc comparisons. The key contrast is T8 (Full Reactor) vs. all other treatments at 12 months. Secondary analysis: Spearman correlations between MS change, Fe_{exch} change, and P_{avail} change across all units at 12 months, testing whether the three-way signature co-varies as the reactor

model predicts. Effect sizes (Cohen's d) will be reported alongside p-values. The minimum detectable effect with n=4 per treatment at α=0.05 and power=0.80 is approximately d=2.0 (large effect), which is consistent with the 3.1× MS enrichment observed in archaeological ADE.

The P sorption isotherm comparison (0 vs 12 months) is the ultimate test of the reactor mechanism. It is analyzed as a Freundlich or Langmuir model fit, comparing maximum adsorption capacity (Q_{max}) and adsorption intensity (K_f) between treatments. A reduction in Q_{max} in T8 relative to T1 would indicate that the reactor has not merely fertilized the soil but has re-engineered its mineralogy—permanently reducing the soil's capacity to fix phosphorus. This is the kinetic triumph described in the companion paper: changing the rate and reversibility of P desorption, not merely the total P stock.

6. Expected Outcomes and Decision Points

Outcome	Interpretation	Next step
T8 shows three-way signature; no other treatment does	Full reactor model validated. All three components necessary and sufficient at incubation scale.	Proceed to pot trial with plants (Phase 2); file provisional patent on amendment formulation; seek USDA/NSF/CGIAR funding for field trials.
T5 (Biochar+Lime) shows partial signature	Biology accelerates but is not strictly necessary; abiotic pH-driven transformation may suffice at elevated pH.	Simplify formulation to two-component amendment; test shelf-stable version.
T8 shows MS increase but no P benefit	Iron transformation occurs but does not release P-fixing capacity; mechanism is real but P benefit requires additional conditions.	Investigate P fractionation; may need longer incubation or different biochar.
No treatment shows significant MS change	Reactor mechanism does not operate at incubation timescale under these conditions.	Revise: test higher inoculant density, waterlogging cycles, or different soil substrate. The hypothesis is falsified for this specific implementation.

Table 4. *Decision matrix linking experimental outcomes to next steps. The experiment is designed to be informative regardless of outcome.*

7. Broader Impact: Carbon and Food Security

If the reactor mechanism can be replicated at field scale, the implications extend far beyond phosphorus retention. The same biochar scaffold that captures iron transformation products sequesters carbon in pyrogenic form resistant to microbial decomposition for centuries to millennia. Unlike carbon capture technologies that compete with productive land use, this intervention *enhances* agricultural productivity while sequestering carbon. The two objectives—greenhouse gas reduction and food security—are achieved as a single integrated intervention rather than competing priorities.

Scale of opportunity: Ferralsols and related acidic tropical soils cover approximately 10^9 hectares, supporting food production for billions of people with chronically low P efficiency. If an engineered terra preta amendment raised P retention from the current ~10–25% efficiency to even half of terra preta's 37%, the aggregate savings in phosphate fertilizer would be on the order of tens of millions of tonnes per year, reducing mining pressure on finite phosphate rock reserves, cutting agricultural runoff that drives aquatic eutrophication, and lowering input costs for smallholder farmers in the tropics.

Carbon arithmetic: Biochar applied at 50 t/ha sequesters approximately 35 tC/ha (at 70% carbon content). Applied to even 1% of global ferralsol area (10 million hectares), this represents 350 million tonnes of stable carbon—roughly 1.3 billion tonnes CO_2-equivalent removed from the atmosphere. The iron transformation further stabilizes the biochar against degradation by forming organo-mineral complexes, potentially extending carbon residence times beyond the already long persistence of pyrogenic carbon alone.

Food security arithmetic: If P use efficiency doubles from 15% to 30% on ferralsols, farmers can achieve the same crop yield with half the fertilizer input, or double yield at the same input. For smallholder farmers spending 30–50% of input costs on phosphate fertilizer, this represents a transformative economic impact. The amendment itself (biochar + lime + inoculant) can be produced locally from agricultural waste, reducing dependence on imported fertilizer supply chains.

A managed ecosystem service: What distinguishes this approach from conventional soil amendment is that the reactor, once initiated, is designed to be self-sustaining. The biochar scaffold persists for millennia. The iron transformation is permanent. The microbial community maintains itself on root exudates and plant litter. The initial application cost is a one-time investment, not an annual input—exactly as archaeological terra preta has demonstrated over 500–2,500 years. This makes the economics fundamentally different from fertilizer: a managed ecosystem service rather than a consumable product. For climate finance frameworks such as the Green Climate Fund, this dual-benefit structure—permanent carbon sequestration coupled with permanent fertility enhancement—represents exactly the kind of integrated intervention that justifies large-scale investment in tropical agriculture.

8. Budget Estimate

Item	Quantity	Unit cost	Total
Ferralsol collection and characterization	200 kg	$500	$500
Biochar (commercial hardwood, 500°C)	15 kg	$3/kg	$45
Agricultural lime	10 kg	$0.50/kg	$5
Microbial inoculant (ATCC culture + media)	1 culture	$500	$500
Pots, trays, greenhouse space (12 mo)	160 pots	$5/pot	$800
Portable MS meter (Bartington MS2 rental or purchase)	1	$3,000	$3,000
Soil chemistry (Mehlich, P, Fe, pH, OC, CEC)	~250 analyses	$25 avg	$6,250
P sorption isotherms	64 samples	$30	$1,920
P fractionation (Hedley)	48 samples	$40	$1,920

Labor / research assistant (12 mo, 0.25 FTE)	12 mo	varies	$5,000–$15,000
Core protocol subtotal			**$18,000–$28,000**
Optional: 16S amplicon sequencing (24 samples)	24	$50	$1,200
Optional: qPCR (80 samples)	80	$15	$1,200
Optional: SEM-EDS (8 samples)	8	$80	$640
Optional: XRD or Mössbauer (4 samples)	4	$200	$800
Full protocol total			**$25,000–$45,000**

Table 5. *Estimated budget. Core protocol is feasible for a university soil science lab or agricultural research station. Optional microbial and mineralogical analyses strengthen mechanistic interpretation.*

9. Timeline

Month	Activity
0–1	Soil collection, baseline characterization, biochar sourcing, inoculant preparation, pot assembly, initial amendment application
1	First destructive sampling (T=1 mo): MS, pH, Fe_{exch}, qPCR
3	Second sampling (T=3 mo): MS, pH, Fe_{exch}, P_{avail}, qPCR, amplicon
6	Third sampling (T=6 mo): Full chemistry suite + P fractionation
9	Data analysis, interim assessment, decision on Phase 2
12	Final sampling: Full chemistry + P sorption isotherm + SEM-EDS + amplicon. Manuscript preparation.
14	Manuscript submission + open data/code deposition

Table 6. *Fourteen-month project timeline from soil collection to manuscript.*

10. Implementation Notes

Lime must react before inoculation. Raw ferralsol at pH 4.0–5.0 is a kill zone for most microbial inoculants. In treatments receiving both lime and inoculant (T7, T8a, T8b), mix the lime into soil and incubate for 14 days at 60% WHC before adding the microbial component. Verify pH has stabilized at ≥5.5 before inoculation. This two-stage approach prevents osmotic shock to the iron-reducing inoculant and mimics the natural sequence in which ash deposition preceded biological colonization in archaeological middens.

Amendment mixing order. (1) Lime into soil, 14-day pre-incubation. (2) Biochar mixed in (pre-wetted to 60% WHC). (3) Inoculant applied as liquid suspension onto biochar-amended soil. (4) First wetting cycle begins. This order ensures the biochar is present as scaffold before the biology is introduced, and the pH environment is hospitable before the microbes arrive.

Budget prioritization. If resources are limited, prioritize measurements in this order: (1) Magnetic susceptibility—non-destructive, zero per-sample cost, most direct indicator of iron transformation. (2) Resin-P or Mehlich-1 P—cheapest P measurement, direct functional endpoint. (3) pH—tracks reactor conditions. (4) Exchangeable Fe—tracks substrate consumption. These four parameters alone, measured at 0 and 12 months on the 36 experimental units, cost under $3,000 in analytical fees and can definitively test the primary hypothesis. The P sorption isotherm, Hedley fractionation, and microbial analyses are mechanistically informative but not required for the initial go/no-go decision.

11. Open Science Commitment

This protocol is released as an open-access document under Creative Commons CC-BY-4.0. All data, analysis code, and results from any implementation will be deposited in a public repository (e.g., Zenodo, Figshare, or GitHub) upon manuscript submission. The protocol is explicitly designed to be replicable by any soil science laboratory with access to a ferralsol, a greenhouse, and standard analytical equipment. Independent replication at multiple sites with different ferralsol substrates is strongly encouraged and would dramatically strengthen the evidence base.

Researchers interested in implementing this protocol are invited to contact the author for collaboration and to register their site in a planned multi-site replication network. Negative results are equally valuable and will be included in any meta-analysis.

12. Potential Funding Sources

Agency / Program	Relevance	Typical award
USDA-NIFA Foundational & Applied Science	Soil health, nutrient management	$150K–$500K
NSF Geobiology & Low-Temperature Geochemistry	Iron biogeochemistry, soil mineralogy	$200K–$400K
CGIAR / One CGIAR Initiatives	Tropical soil fertility, smallholder agriculture	Varies
Bill & Melinda Gates Foundation	Smallholder productivity in sub-Saharan Africa	$100K–$1M+
DOE Biological & Environmental Research	Carbon sequestration in soils	$200K–$500K
Brazilian FAPESP / FAPEAM	Amazonian soil science, ADE research	R$100K–R$500K
EU Horizon Europe (Soil Mission)	Soil health, carbon farming	€150K–€2M
Green Climate Fund (GCF)	Climate-smart agriculture, carbon sequestration in Global South	$500K–$10M+
USAID Feed the Future Innovation Labs	Soil fertility and food security in developing regions	$50K–$500K

Table 7. *Potential funding sources aligned with the project scope. The core protocol ($18–28K) is feasible as a seed grant or pilot project within a larger proposal.*

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